

6. Budget Plan:

Overhead
Other

Date:

March 14, 1956

51% on all items

1. Name of Investigator: 1. **Paul S. Larson, Ph. D.** 2. **H. B. Haag, M.D.**
3. **Herbert McKennis, Ph.D.**
3. Title: 1. **Professor of Pharmacology** 2. **Professor of Pharmacology**
3. **Professor of Research Pharmacology**
3. Institution: **Medical College of Virginia**
4. Project or Subject: **Enzymatic Transformations of Nicotine**
5. Additional Requirements:

[illegible]

5. Detailed Plan of Procedure (Use reverse side if additional space is needed):

Numerous experiments on animals have shown that only a small percent of administered nicotine is excreted unchanged (Larson, Ind. Eng. Chem. 41, 279, 1952). However, studies with C¹⁴ labeled nicotine show that virtually all of the C¹⁴ is excreted in the urine within 36 hours (Ganz, Kelsey and Gelling, J. Pharmacol. & Exper. Therap. 103, 209, 1951; Bennett, Tedeschi & Larson, Arch. Int. Pharmacodyn. 98, 221, 1954). Recent studies in this laboratory on urine from dogs receiving C¹⁴ labeled nicotine indicate the presence of three major and possibly four minor metabolites of nicotine (Owen & Larson, Fed. Proceedings 14, 376, 1955). The chemical nature of these biotransformation products is currently being investigated.

To complement these in vivo studies on nicotine metabolism, it is proposed to initiate in vitro studies directed toward elucidating the enzyme systems involved. Since early studies in which tissue slices were used, no investigations on the enzymatic transformation of nicotine by animals have been recorded (Weib et al., Biochem. Z. 308, 355, 1941; *ibid*, 318, 531, 1948; Annal. d. Chem. 562, 60, 1949). These studies provide little information other than a few characteristics of the systems involved.

Data on possible intermediates in the metabolism of nicotine being obtained in our laboratory, together with general advances in enzymology, make possible a study in systems less complex than whole tissue slices. For example, purified preparations fortified with co-factors according to modern enzymatic techniques should yield new information. The fact that possible intermediates have been disclosed also makes possible new studies on the effect of oxidation - reduction and other type enzymes on these compounds. Results from the enzymatic studies would be compared to intact animal studies using various species. C14 labeled nicotine will be used in experiments where such labeling is desirable.

J. T. A. [illegible]
Business Officer of the Institution

1003541861

6. Budget Plan:

Salaries	\$13,000
Expendable Supplies	4,000
Permanent Equipment	10,050
Overhead	1,730
Other	300
Total	\$29,080

*10% on all items excepting permanent equipment.

7. Anticipated Duration of Work: April 2, 1964 - May 2, 1964.

3. Barbara McGovern, Ph.D.

Three years

8. Facilities and Staff Available: Department of Microbiology Department of Pharmacology

General pharmacologic and biochemical equipment and laboratory. Lardy-type Warburg apparatus, refrigerated centrifuge, ^{14}C counting equipment (Libby anticoincidence counter, Nuclear Instruments gas-flow counter with automatic sample changer), paper chromatographic equipment and electrolytic desalter, greenhouse facilities for growing ^{14}C labeled plants

Staff: Part-time services of - Dr. Paul S. Larson
Dr. H. B. Haug
Dr. Herbert McKennis

9. Additional Requirements:

What, if any, there may be will depend on how the project develops.

10. Additional Information (Including relation of work to other projects and other sources of supply).

The breakdown of permanent equipment items is as follows: Perkin Elmer Spectracord 4000 with attachments \$8400, thermocap \$78, circulating pump \$42, Senior Isotemp Oven \$240, International Centrifuge Size No. 2 with attachments \$960, Welch vacuum pump 1402 VED with guard \$310. The major item of the Spectracord is needed for reaction rate studies, identification studies, etc., in this and in the other programs underway in this laboratory involving tobacco and its constituents.

The salary item is intended for stipends for two additional professional scientists to be added to the staff; one at the M.S. level (\$5,500), the other at the Ph.D. level (\$7,500).

In view of the need from the start of this project for the permanent equipment items, it is requested that if this application is granted that the initial payment will be for \$15,000.

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Many reasonable intermediates in the metabolism of steroids being obtained in our laboratory, together with general advances in steroid chemistry, make possible a study in several less complex, but whole tissue slices. /s/ Paul S. Larson,
fertilize with reductants, monitoring in vitro enzyme /s/ E. B. Mag - these preparations
information. The fact that possible /s/ Herbert McKenna Jr.
possible intermediates in the action of synthesis - reduction and other type enzymes on
these compounds. Results from the enzymatic studies would be required to intact
animal studies using radioisotopes. All labeled steroids will be used in experi-
ments where such labeling is desirable. /s/ W. E. Tompkins, Controller

1/s/ Paul S. Larson

/s/ H. B. Hall

/s/ Herbert McKenna Jr

Director of Project

/s/ W. E. Tompkins, Comptroller

Business Officer of the Institution

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